Progress in Natural Science: Materials International 31 (2021) 541–545

HOSTED BY

Contents lists available at ScienceDirect

Progress in Natural Science: Materials International

journal homepage: www.elsevier.com/locate/pnsmi

Original Research

Nanoengineered polypeptides from tetraphenylethylene-functionalized N-carboxyanhydride: Synthesis, self-assembly and intrinsic aggregation-induced emission

Xu Wang [a,b,1], Zihao Zhao [a,c,1], Tianrui Xue [a], Zhengzhong Tan [a], Ziyuan Song [a], Shiqi Wei [a], Yang Bo [a], Ying Wang [a], Jianjun Cheng [a,*]

[a] Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, 61801, United States
[b] Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics (Theranostics), School of Pharmacy, Tianjin Medical University, Tianjin, 300070, PR China
[c] School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, No. 800 Dongchuan Rd., Minhang District, Shanghai, 200240, PR China

ARTICLE INFO

Keywords:
N-carboxyanhydride
Ring-opening polymerization
Polypeptide
Micelle
Aggregation-induced emission

ABSTRACT

Ring-opening polymerization of *N*-carboxyanhydrides (NCAs) bearing pendant groups creates functional polypeptides. In this paper, we report the design, synthesis and polymerization of tetraphenylethylene (TPE)-modified NCA, which is used to incorporate aggregation-induced emission (AIE)-active segments into polypeptides. Specifically, we attempted the synthesis of amphiphilic methoxy poly(ethyleneglycol)-*block*-poly(γ-benzyl-$_L$-glutamate)-*block*-poly(γ-4-(1,2,2-triphenylvinyl)benzyl-$_L$-glutamate) (PEG-PBLG-PTPELG), which had well-controlled molecular weight and narrow polydispersity. The hydrophilic PEG and hydrophobic PBLG-PTPELG assembled into micelles in aqueous solution with diameter around 70 nm, displaying AIE at 480 nm. Our work demonstrates that the TPE-modified NCA is applicable for the preparation of the AIE-active polypeptides, integrating the AIE luminogens into the polypeptides which may offer unique opportunities for tuning AIE properties by adjusting the composition and conformation of polypeptides. We preliminarily explored the use of the amphiphilic polypeptides for the formulation of doxorubicin-containing micelles that can potentially be used for real-time monitoring of the nanomedicine distribution.

1. Introduction

Polymeric micelles have been intensively explored for their applications in drug delivery given that their ease of preparation via self-assembly, tunable composition and facile functionalization [1–4]. The delivery efficiency of the micelles varies drastically from one material or system to another, and is determined by their dynamic behavior and stability in the biological systems. Tracking and analysis of the self-assembled micelles in the process of drug delivery could be very difficult. Fluorescence labeling of micelles has been one of the key techniques to investigate real-time drug delivery process. However, aggregation-caused quenching effect of traditional dyes significantly restrains the accurate assessments [5–7].

Aggregation-induced emission (AIE) probes luminesce weakly in good solvents but could emit intensive fluorescence in the aggregated state, enabling the imaging in complicated matrix [8–11]. AIE luminogens (AIEgens) including tetraphenylethylene- (TPE), hexaphenylsilole- (HPS) or distyreneanthracene- (DSA) derivatives have been intensively investigated in the past few years [12–15]. Great efforts have been devoted to developing AIE-active polymers via non-covalent encapsulation strategies or covalent-grafting strategies [16–18]. Loading hydrophobic AIE molecules into polymers via non-covalent interactions may not avoid their premature leakage. Covalent tethering of AIEgens onto polymers is presumably a better alternative to achieve AIE-active polymers with stably incorporated fluorescence property. However, the process of conjugating AIEgens to pre-existing polymers is not always easy and feasible, due to compatibility issue of the AIEgens and polymers, laborious bioconjugation procedures, and poor conjugation efficiency.

Ring-opening polymerization (ROP) of functional *N*-carboxyanhydrides (NCAs) is a facile and reliable strategy to fabricate polypeptides with desired architectures, quantitative function groups and narrow polydispersity index (PDI) [19–21]. The synthesis of AIEgen-based NCA has been rarely explored. Herein, we attempted the design and synthesis

* Corresponding author.
E-mail address: jianjunc@illinois.edu (J. Cheng).
[1] These authors contributed equally.

https://doi.org/10.1016/j.pnsc.2021.03.003
Received 17 October 2020; Received in revised form 8 March 2021; Accepted 19 March 2021
Available online 3 July 2021
1002-0071/© 2021 Chinese Materials Research Society. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

of a TPE modified NCA (γ-4-(1,2,2-triphenylvinyl)benzyl-$_L$-glutamate NCA (TPELG-NCA)) derivatized from TPE-glutamic acid conjugates (Scheme 1) and its use of synthesizing amphiphilic AIE-active polypeptides with stably conjugated TPE. Specifically, methoxy poly(ethylene glycol)-*block*-poly(γ-benzyl-$_L$-glutamate) amine (PEG-PBLG), an amphiphilic di-block copolymer bearing an amino terminus, was used as the macroinitiator to trigger the ROP of TPELG-NCAs in water-in-oil (w/o) emulsion, forming methoxy PEG-PBLG-*block*-poly(γ-4-(1,2,2-triphenylvinyl)benzyl-$_L$-glutamate) (PEG-PBLG-PTPELG) via the SIMPLE polymerization method that we developed recently (Scheme 1) [22]. The synthesized PEG-PBLG-PTPELG self-assembled into micelles in aqueous solution, emitting strong blue fluorescence (480 nm) of TPE under the irradiation at 365 nm. TPEs involved in the core of micelles afforded π-π stacking with hydrophobic drugs, such as doxorubicin (DOX). The AIE at 480 nm from TPE and the emission at 600 nm from DOX may enable dual-fluorescence real-time tracking and analysis of the polypeptide micelle drug delivery system in the biological systems.

2. General experimental details

2.1. Materials

All reagents were purchased from Milliore Sigma (St. Louis, MO, USA) unless otherwise specified. Amino acids were provided by Chem-Impex International, Inc. (Wood Dale, IL, USA). 2-Bromo-1,1,2-triphenylethylene, *N*,*N*′-dicyclohexylcarbodiimide (DCC), 4-Dimethylaminopyridine (DMAP) and trifluoroacetic acid (TFA) were obtained from TCI America (Portland, OR, USA). Methoxy poly(ethylene glycol) amine (mPEG-NH_2, 5 kDa) was purchased from Laysan Bio, Inc. (Arab, AL, USA). Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Anhydrous tetrahydrofuran (THF) and hexane were dried by a column packed with alumina. Dichloromethane (DCM) was prepared by refluxing over CaH_2 for 24 h, distilling and purging with nitrogen gas for 15 min. Anhydrous *N*, *N*-dimethylformamide (DMF) was treated with polymer-bound isocyanates (MilliporeSigma, St. Louis, MO, USA) to remove any suspicious amine residues and was stored at −40 °C in a glovebox system. BCP buffers containing boric acid, citric acid, and sodium phosphate as active species (pH 3 and pH 7) were used in the experiments.

2.2. Instrumentation

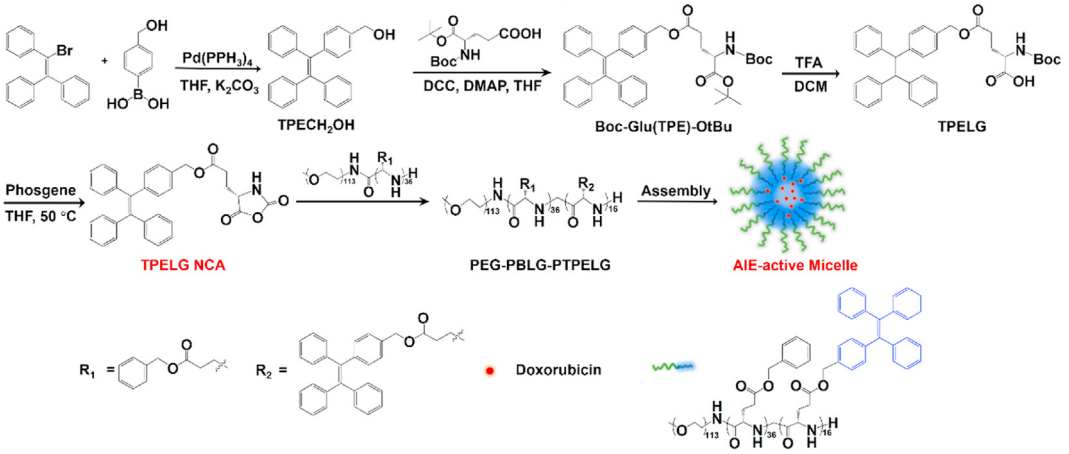
Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Varian VXR500 (500 MHz) spectrometer. MestReNova (version 12.0.3, Mestrelab Research, Escondido, CA, USA) was used in the analysis of NMR data. Gel permeation chromatography (GPC) data were collected via an instrument equipped with an isocratic pump (1260 Infinity II, Agilent, Santa Clara, CA, USA), a multi-angle static light scattering (MALS) detector operating at a wavelength of 658 nm (DAWN HELEOS-II, Wyatt Technology, Santa Barbara, CA, USA), and a differential refractometer (DRI) detector (Optilab T-rEX, Wyatt Technology, Santa Barbara, CA, USA). A serial of size exclusion columns (PLgel MIXED-B columns, 10 μm, 7.5 × 300 mm, Agilent, Santa Clara, CA, USA) were used in separation, which were maintained at a temperature of 40 °C using DMF containing 0.1 M LiBr as eluting agent at a flow rate of 0.7 mL/min. Samples were filtered through a 0.45 μm PTFE filter before analysis. Absolute molecular weights of polypeptides were determined based on the *dn*/*dc* value processed by the software ASTRA 7 (version 7.1.3.15, Wyatt Technology, Santa Barbara, CA, USA). Fourier transform infrared (FTIR) spectra were obtained using a PerkinElmer 100 serial FTIR spectrophotometer (PerkinElmer, Santa Clara, CA, USA). High performance liquid chromatography (HPLC) spectra were obtained by a system equipped with an isocratic pump (1260 Infinity II, Agilent, Santa Clara, CA, USA) and a detector with the wavelength from 190 nm to 400 nm (DAWN HELEOS-II, Wyatt Technology, Santa Barbara, CA, USA).

2.3. Synthesis of 4-(1,2,2-triphenylvinyl)phenyl)methanol ($TPECH_2OH$)

The reaction was carried out by following previously reported procedures [23]. 2-Bromo-1,1,2-triphenylethylene (1.0 g, 3.0 mmol) and 4-(hydroxymethyl)phenylboronic acid (0.55 g, 3.6 mmol) were dissolved in 40 mL THF, to which 20 mL potassium carbonate aqueous solution (2 M) was added. After the mixture was stirred for 0.5 h at room temperature under N_2, tetrakis (triphenylphosphine) palladium (0) (0.010 g) was added. After degassing by three freeze-pump-thaw cycles to remove oxygen, the reaction was refluxed under dean stark trap overnight. Followed by removing the solvent under reduced pressure, the crude product was purified by chromatography with DCM as the eluent to obtain $TPECH_2OH$ (1.07 g, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.06 (d, 19H), 4.61 (s, 2H).

2.4. Synthesis of γ-TPE-$_L$-glutamate acid (TPELG)

Boc-$_L$-glutamic acid-γ-TPE ester-α-tert-butyl ester (Boc-Glu(TPE)-OtBu) was prepared by following a published protocol [24]. $TPECH_2OH$ (0.54 g, 1.5 mmol), Boc-$_L$-glutamic acid-α-tert-butyl ester (Boc-Glu-OtBu) (0.91 g, 3 mmol), DCC (2.08 g) and DMAP (0.12 g) were separately added and dissolved in THF (30 mL), which was stirred at room temperature overnight. After quenching by 20 mL saturated ammonium chloride aqueous solution, the organic phase was washed three times by saturated brine and dried by anhydrous sodium sulfate overnight. The obtained crude product was purified twice by chromatography with



Scheme 1. Schematic illustration of synthesis of TPELG NCA and PEG-PBLG-PTPELG.

hexane/EtOAc (v/v = 1/1), followed by EtOAc as eluting agents to get Boc-Glu(TPE)-OtBu (0.57 g, 58% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.06 (d, 18H), 5.04 (d, 2H), 4.20 (s, 1H), 2.43 (s, 2H), 2.17 (s, 1H), 1.93 (s, 1H), 1.44 (d, 20H).

To get TPELG, Boc-Glu(TPE)-OtBu (0.26 g, 0.4 mmol) was re-dispersed in 5 mL DCM and then cooled down to 0 °C. A mixture of TFA (5 mL) and DCM (5 mL) was added dropwise under stirring at 0 °C. After stirring for another 4 h, the solvent was removed under reduced pressure. The resulting solid was recrystallized from anhydrous diethyl ether to give TPELG (0.14 g, 71% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.03 (s, 19H), 4.94 (s, 2H), 4.11 (s, 1H), 2.59 (s, 2H), 2.23 (d, 2H).

2.5. Synthesis of TPELG NCA

TPELG (98 mg, 0.2 mmol) was added into a flame-dried vial (20 mL) and dried under vacuum for more than 2 h. Anhydrous THF (2 mL) was then pipetted in the vial to create a suspension, then cooling on ice. Phosgene/toluene solution (220 μL, 15 wt%, 0.3 mmol of phosgene, 1.5 eq.) was added and the mixture became clear instantly. The vial was then placed in a prewarmed oil bath at 50 °C, heating for 15 min. After removing the solvent and residual phosgene under vacuum, the crude product was dissolved in anhydrous THF and precipitated by cold hexane/ether (1:1, v/v) for three times, during which the precipitate was separated by a centrifuge. The TPELG NCA (94.6 mg, 91% yield) was stored at −40 °C in a glovebox system for further use in ROP. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.99 (d, 19H), 6.07 (s, 1H), 4.98 (s, 2H), 4.28 (s, 1H), 2.52 (s, 2H), 2.21 (s, 1H), 2.04 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 169.5, 144.3, 143.7, 140.4, 133.2, 131.7, 131.4, 127.9, 126.7, 67.1, 57.2, 30.2, 27.1. HR-MS: *m/z* calculated for [M + Na]: 540.1787; found: 540.1796.

2.6. Synthesis and self-assembly of PEG-PBLG-PTPELG

Purified γ-benzyl-$_L$-glutamate *N*-carboxyanhydride (BLG-NCA) was synthesized and purified by following reported procedures [25]. Methoxy poly(ethylene glycol)-*b*-poly(γ-benzyl-$_L$-glutamate) amine (PEG-PBLG), behaving as macroinitiators as well as emulsion stabilizers in SIMPLE ROP procedure, was prepared according to literature procedures [22]. Briefly, TPELG NCA (70.4 mg, 0.136 mmol) was dissolved in 8 mL DCM, purified by centrifugation at 8, 000 rpm for 3 min (25 °C), and then washed with BCP buffer (pH = 3.0, 200 μL, 18 wt%). PEG-PBLG macroinitiator dissolved in DCM (800 μL, 1 mM) was mixed up with BCP buffer (pH = 7.0, 24 μL, 2 wt%) via vortex to form emulsion. This emulsion was then mixed with TPELG NCA solution (800 μL, 17 mM) to trigger the polymerization.

The PEG-PBLG-PTPELG re-dissolved in DMF (1.0 mL, 5 mg/mL) was put in a 7 mL vial, followed by pumping 4 vol of DI water into the solution by a syringe pump (KD Scientific, Holliston, MA, USA. Addition speed: 0.1 mL/min) under stirring. The suspension was stirred for another 2 h at room temperature and then transferred to a dialysis bag (MWCO = 1 kDa) for purification.

2.7. Formulation of DOX-loaded PEG-PBLG-PTPELG micelles

Nanoprecipitation was used to load DOX [26]. PEG-PBLG-PTPELG (5 mg) and DOX (0.25, 0.50 and 1.00 mg, respectively) were dissolved in 1.0 mL DMF. The solution was added dropwise into 4 vol of DI water in a 7 mL vial by a syringe pump under stirring. The suspension was stirred for another 6 h at room temperature and then transferred to a dialysis bag (MWCO = 1 kDa) for purification. After lyophilization, the DOX-loaded PEG-PBLG-PTPELG micelles were collected for further experiments.

2.8. Drug release property of DOX-loaded PEG-PBLG-PTPELG micelles

The DOX-loaded PEG-PBLG-PTPELG micelles were re-suspended in FBS/PBS buffer (1 mL, 1:1, v/v) and transferred into a dialysis tube (MWCO 1kD). Immerge the dialysis tube in a tube containing 4 mL of PBS buffer at 37 °C under constant shaking. At 0, 1, 2, 4, 8, 12, 24 h, 1.0 mL of the solution outside the dialysis tube was drawn out for the measurement of DOX concentration and equal volume of fresh PBS buffer was supplemented for further experiments. The DOX concentration was measured by HPLC with a fluorescence detector (Ex: 470 nm, Em: 595 nm).

3. Results and discussion

Self-assembled polymeric micelle enables to load hydrophobic drugs in the core and to enhance the colloidal stability based on its hydrophilic shell in physiological fluids. It is vital to monitor the dynamic behaviors of polymeric micelles for the best treatment outcomes. AIEgens with intensive fluorescence in the aggregated state are recognized as a promising labeling probe, which differ from normal fluorescence dyes suffering quenching in high concentration. We thus designed a TPE-modified NCA to prepare AIE-active polypeptides, which are attached with quantitative AIEgens groups, and display stable AIE luminescence when assembled into micelles.

3.1. Synthesis and characterization of TPELG-NCA

To start the synthesis of TPE-modified NCA, a hydroxyl group was introduced to TPE molecule by conjugating 2-bromo-1,1,2-triphenylethylene with 4-(hydroxymethyl)phenylboronic acid via Suzuki chemistry [23], which functioned as an active unit to react with amino acid (Scheme 1). The resulting TPECH_2OH was grafted onto Boc-Glu-OtBu according to Tai et al. [24], followed by the removal of protection groups in TFA and DCM (1:1, v/v), obtaining the desired conjugates (TPELG) (Figs. S1–S3 in the Supporting Information). Phosgenation of TPELG generated the target product of TPELG-NCA with pendant TPE group at γ-COOH position. The monomers were precipitated in cold hexane/ether in a high yield of 91%. ^{1}H, ^{13}C NMR and MS analyses were carried out to verify the successful synthesis of TPELG-NCA (Fig. 1, Figs. S4 and S5 in the Supporting Information). A characteristic peak at 6.99 ppm demonstrated the presence of TPELG skeleton in the as-prepared compound. The peaks at 6.07 and 4.28 ppm were ascribed to the protons in hydroxyl groups of NCAs (Fig. 1A). All these peaks indicated the successful synthesis of TPELG-NCA, confirming the result obtained from MS analyses ([M + Na]: 540.1796) (Fig. S5 in the Supporting Information). The purified TPELG-NCA was stable in moisture-free conditions and stored at −40 °C in a glovebox system for more than 3 months with no obvious change.

3.2. ROP of TPELG-NCA

A SIMPLE strategy we developed recently was used in the ROP of TPELG-NCA, allowing controlled polymerization in an open-air system [22]. PEG-PBLG, an amphiphilic di-block copolymer bearing an amino terminus, was used as the macroinitiator to trigger the ROP of TPELG-NCA in water/DCM emulsion. Given the strong tendency of PEG-PBLG to localize at the water/DCM interface, the PEG segments acted as the designated interfacial anchors, while the hydrophobic PBLG segments folded to α-helical arrays protruding into DCM phase as the macroinitiators. The water-in-oil (w/o) emulsion by sonicating an aqueous buffer (pH = 7.0) and a DCM solution of PEG−PBLG, was then mixed with an equal volume of purified TPELG-NCA in DCM to start the polymerization ($[M]_0$ = 50 mM, $[I]_0$ = 0.5 mM). The polymerization monitored by FT-IR indicated the complete generation of PEG-PBLG-PTPELG in 2.5 h, along with the disappeared characteristic peaks of NCAs at 1780 and 1850 cm^{-1} (Fig. 1C). Further evidence of the succeeded synthesis of PEG-PBLG-PTPELG was given in ^{1}H NMR spectrum, showing two peaks (6.97 and 7.04 ppm) ascribed to P_2 hydrogens of TPE groups (Fig. 1B). GPC analysis of PEG-PBLG-PTPELG revealed a monodispersed peak with an obtained MW M_n = 20.8 kDa (Fig. 1D). ROP of TPELG-NCAs proceeded to

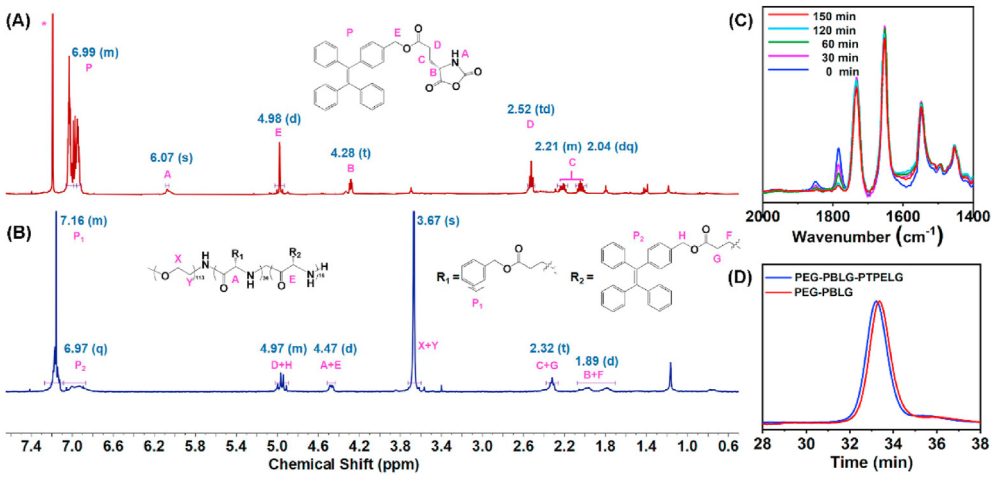


Fig. 1. Characterization of TPELG-NCA and PEG-PBLG-PTPELG. (A–B) ^{1}H NMR spectrum (500 MHz) of (A) TPELG-NCA and (B) PEG-PBLG-PTPELG in $CDCl_3$/TFA-d_6 (85:15, v/v); (C) FTIR spectra showing polymerization of TPELG-NCAs in water/DCM (water: DCM = 1:100) triggered by PEG-PBLG macroinitiator; (D) Normalized GPC-LS spectra of PEG-PBLG and PEG-PBLG-PTPELG.

yield predictable polypeptides in complete monomer conversion with no evidence of inactive chains in GPC spectra (Fig. 1D). The MW of PEG-PBLG-PTPELG compared to that of PEG-PBLG was increased by 7.8 kDa, which indicates PTPELG with a degree of polymerization (DP) of 16, conforming well with the designed DP. The dispersity (Đ = Mw/Mn) of the resulting polypeptides was low (1.12), demonstrating good control throughout the polymerization process. All the data show that the TPELG-NCAs are able to undergo polymerization when initiated with PEG-PBLG, providing a facile access to a range of TPE-functionalized polypeptides with tailored molecular weights and functionalities.

3.3. Self-assembly of PEG-PBLG-PTPELG

Amphiphilic PEG-PBLG-PTPELG with both hydrophilic and hydrophobic segments can self-assemble into micelles in aqueous phase. To verify the structure-dependent AIE of PEG-PBLG-PTPELG, micellar nanoparticles were induced by adding DI water dropwise into the DMF solution of PEG-PBLG-PTPELG (Fig. 2). As expected, there was no obvious AIE luminescence of PEG-PBLG-PTPELG observed in the benign solvent of DMF. In contrast, PEG-PBLG-PTPELG displayed strong blue fluorescence in water/DMF bi-solvent system under irradiation with a 365 nm UV lamp, due to the concentrated AIEgens in the core of assembled micelles. Dynamic light scattering (DLS) measurement demonstrated a main sharp peak at around 70 nm, indicating the formation of PEG-PBLG-PTPELG micellar nanoparticles with low size distribution. Another broad peak centered at 370 nm resulted from part of aggregated micellar nanoparticles (Fig. 2A). The characteristic AIE (480 nm) of pendant TPE groups in PEG-PBLG-PTPELG was further verified in fluorescence spectra, which possesses potential applied in bioimaging or biosensing (Fig. 2C).

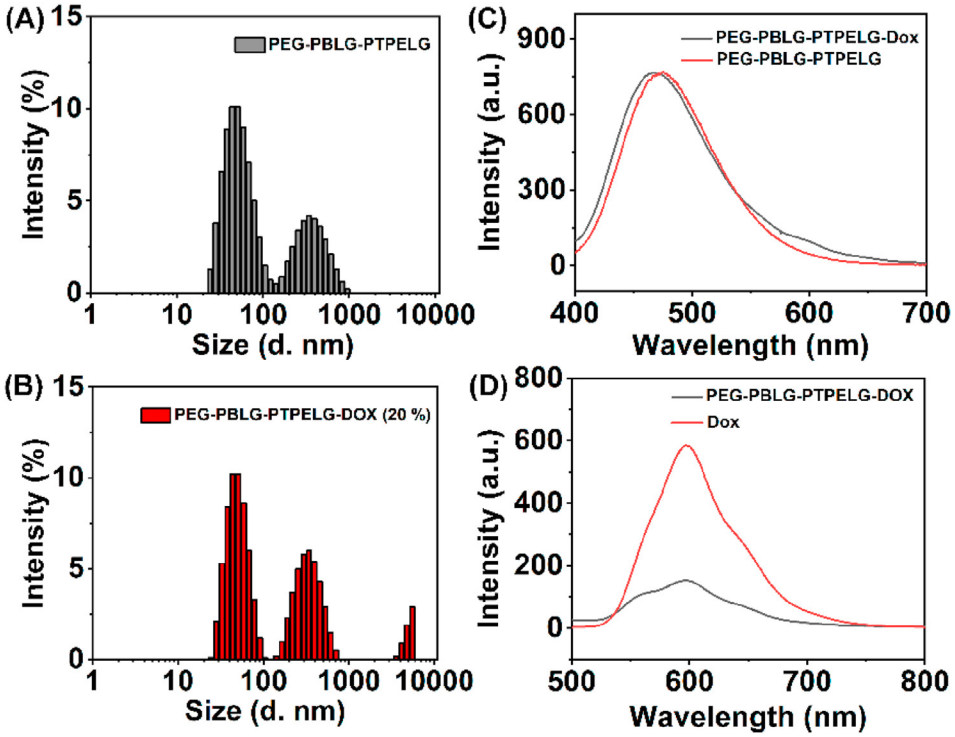


Fig. 2. Preparation and characterization of DOX-loaded AIE-active polypeptide. (A, B) DLS results of PEG-PBLG-PTPELG (A) and PEG-PBLG-PTPELG-DOX (20% feeding amount of DOX) (B) NPs in DMF/water (10 μg/mL); (C) Fluorescence spectra of PEG-PBLG-PTPELG-DOX and PEG-PBLG-PTPELG NPs in DMF/water under 365 nm UV irradiation (100 μg/mL); (D) Fluorescence spectra of PEG-PBLG-PTPELG-DOX NPs and DOX in DMF/water under 440 nm irradiation (100 μg/mL).

3.4. Formulation of DOX-loaded PEG-PBLG-PTPELG micelles

DOX, as a model hydrophobic drug, was mixed with PEG-PBLG-PTPELG to prepare drug-loaded micelles through nanoprecipitation method [26]. The feeding mass ratio of DOX: PEG-PBLG-PTPELG were varied from 1:20 (5 wt%) to 1:5 (20 wt %) to determine the drug loading capacity (DLC, wt%) and drug encapsulation efficiency (DEE, %). Quantitative measurements based on HPLC revealed the increase in DLC from 0.5- to 2.7-wt% along with the elevated feeding ratio. A maximum DLC of DOX (2.7 wt%) in the studied range was achieved when the mass ratio was fixed as 20 wt%, with a DEE of 13.4%, indicating the succeeded loading of DOX in micelles through hydrophobic/aromatic interactions with the PBLG-PTPELG components of the polypeptides (Table S1 in supporting information). A similar trend was observed in DLS analyses (Fig. 2, Fig. S6 and Table S1 in supporting information). The dynamic diameter of PEG-PBLG-PTPELG micelles was increased from 70 nm to 80 nm after loading DOX (5 wt%) into micelles (Fig. 2A and B). Further increase of the feeding ratio from 5- to 20-wt% led to a slight increment in DLS (Fig. S6 and Table S1 in supporting information). Compared to PEG-PBLG-PTPELG micelles, the size distribution of DOX-loaded micelles exhibited a triphasic trend with a smaller diameter particle distribution accompanied by two larger diameter particles distributions (Fig. 2 and Fig. S6 in supporting information). An additional peak around 3000 nm was observed after DOX loading, resulting from the aggregation of unencapsulated DOX due to its high hydrophobicity.

In addition, the formed DOX-loaded PEG-PBLG-PTPELG micelle emitted dual-fluorescence at 480 nm and 600 nm by two excitation pathways, AIE luminescence (480 nm) from TPE groups and fluorescence (600 nm) from DOX molecules (Fig. 2C and D and Fig. S7), which enabled to monitor the delivery dynamics of micelles and to trace the release of DOX in the biosystems by dual-fluorescence imaging [27,28]. To clarify the drug release property, the drug release behavior of the DOX-loaded micelles was investigated in PBS buffer (Fig. S8). The time-dependent drug release curve showed a quick release (82.9% of DOX) in the initial 3 h. A platform of drug release was achieved for a prolonged period of time, generating a stable drug concentration, which showed a typical two-phase-release property of micelle-based drug delivery systems [29,30].

4. Conclusions

We designed and synthesized TPELG-NCA for ROP to prepare TPE-conjugated polypeptides. The ratio of TPE to polypeptides can be precisely controlled. The resulting amphiphilic polypeptide, PEG-PBLG-PTPELG, self-assembles into micelles in water/DMF with instinct AIE at 480 nm. The DOX loaded in the core of micelles gives another emission at 600 nm and the resulting micelle offers dual fluorescence signals. This approach provides an alternative strategy to fabricate self-luminescence theranostic micelles, which may enable stably and controlled incorporation of AIEgens to polymeric micelles for potential tracking of the behavior and changes of micelles in *vitro* and in *vivo*.

Author information

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

X.W. acknowledges the support from National Natural Science Foundation of China (No. 21974095) and the State Scholarship Fund of China Scholarship Council (No. 201706940015). This work was supervised by J.C. while X.W. and Z.Z. were working at University of Illinois as visiting scholars.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnsc.2021.03.003.

References

[1] S.P. Chali, B.J. Ravoo, Angew. Chem. Int. Ed. 59 (2020) 2962–2972, https://doi.org/10.1002/anie.201907484.
[2] Y.C. Chang, Y. Jiao, H.E. Symons, J.F. Xu, C.F.J. Faul, X. Zhang, Chem. Soc. Rev. 48 (2019) 989–1003, https://doi.org/10.1039/c8cs00806j.
[3] H. Cabral, K. Miyata, K. Osada, K. Kataoka, Chem. Rev. 118 (2018) 6844–6892, https://doi.org/10.1021/acs.chemrev.8b00199.
[4] N. Kamaly, B. Yameen, J. Wu, O.C. Farokhzad, Chem. Rev. 116 (2016) 2602–2663, https://doi.org/10.1021/acs.chemrev.5b00346.
[5] C. Wang, W.P. Fan, Z.J. Zhang, Y. Wen, L. Xiong, X.Y. Chen, Adv. Mater. 31 (2019) 1904329, https://doi.org/10.1002/adma.201904329.
[6] H. Kim, G. Kwak, K. Kim, H.Y. Yoon, I.C. Kwon, Biomaterials 213 (2019) 119207, https://doi.org/10.1016/j.biomaterials.2019.05.018.
[7] J.C. Li, J.H. Rao, K.Y. Pu, Biomaterials 155 (2018) 217–235, https://doi.org/10.1016/j.biomaterials.2017.11.025.
[8] J.D. Luo, Z.L. Xie, J.W.Y. Lam, L. Cheng, H.Y. Chen, C.F. Qiu, H.S. Kwok, X.W. Zhan, Y.Q. Liu, D.B. Zhu, B.Z. Tang, Chem. Commun. (2001) 1740–1741, https://doi.org/10.1039/b105159h.
[9] X.L. Cai, B. Liu, Angew. Chem. Int. Ed. 59 (2020) 9868–9886, https://doi.org/10.1002/anie.202000845.
[10] D. Wang, B.Z. Tang, Acc. Chem. Res. 52 (2019) 2559–2570, https://doi.org/10.1021/acs.accounts.9b00305.
[11] D. Ding, K. Li, B. Liu, B.Z. Tang, Acc. Chem. Res. 46 (2013) 2441–2453, https://doi.org/10.1021/ar3003464.
[12] J. Liang, B. Tang, B. Liu, Chem. Soc. Rev. 44 (2015) 2798–2811, https://doi.org/10.1039/c4cs00444b.
[13] X.G. Gu, R.T.K. Kwok, J.W.Y. Lam, B.Z. Tang, Biomaterials 146 (2017) 115–135, https://doi.org/10.1016/j.biomaterials.2017.09.004.
[14] J. Qian, B.Z. Tang, Chem. 3 (2017) 56–91, https://doi.org/10.1016/j.chempr.2017.05.010.
[15] Y.C. Chen, J.W.Y. Lam, R.T.K. Kwok, B. Liu, B.Z. Tang, Mater. Horiz. 6 (2019) 428–433, https://doi.org/10.1039/c8mh01331d.
[16] R. Hu, A.J. Qin, B.Z. Tang, Prog. Polym. Sci. 100 (2020) 1600433, https://doi.org/10.1016/j.progpolymsci.2019.101176.
[17] A. Qin, J.W.Y. Lam, B.Z. Tang, Prog. Polym. Sci. 37 (2012) 182–209, https://doi.org/10.1016/j.progpolymsci.2011.08.002.
[18] J. Li, J.X. Wang, H.X. Li, N. Song, D. Wang, B.Z. Tang, Chem. Soc. Rev. 49 (2020) 1144–1172, https://doi.org/10.1039/c9cs00495e.
[19] Z.Y. Song, H.L. Fu, R. Wang, L.A. Pacheco, X. Wang, Y. Lin, J.J. Cheng, Chem. Soc. Rev. 47 (2018) 7401–7425, https://doi.org/10.1039/c8cs00095f.
[20] Z.Y. Song, Z.Y. Han, S.X. Lv, C.Y. Chen, L. Chen, L.C. Yin, J.J. Cheng, Chem. Soc. Rev. 46 (2017) 6570–6599, https://doi.org/10.1039/c7cs00460e.
[21] Z.Y. Song, Z.Z. Tan, J.J. Cheng, Macromolecules 52 (2019) 8521–8539, https://doi.org/10.1021/acs.macromol.9b01450.
[22] Z.Y. Song, H.L. Fu, J. Wang, J.S. Hui, T.R. Xue, L.A. Pacheco, H.Y. Yan, R. Baumgartner, Z.Y. Wang, Y.C. Xia, X.F. Wang, L.C. Yin, C.Y. Chen, J. Rodriguez-Lopez, A.L. Ferguson, Y. Lin, J.J. Cheng, Proc. Natl. Acad. Sci. U.S.A. 116 (2019) 10658–10663, https://doi.org/10.1073/pnas.1901442116.
[23] H.Y. Li, X.Q. Zhang, X.Y. Zhang, B. Yang, Y. Yang, Y. Wei, Polym. Chem. 5 (2014) 3758–3762, https://doi.org/10.1039/c4py00386a.
[24] W.Y. Tai, R. Mo, Y. Lu, T.Y. Jiang, Z. Gu, Biomaterials 35 (2014) 7194–7203, https://doi.org/10.1016/j.biomaterials.2014.05.004.
[25] J.R. Kramer, T.J. Deming, Biomacromolecules 11 (2010) 3668–3672, https://doi.org/10.1021/bm101123k.
[26] J. Cheng, B.A. Teply, I. Sherifi, J. Sung, G. Luther, F.X. Gu, E. Levy-Nissenbaum, A.F. Radovic-Moreno, R. Langer, O.C. Farokhzad, Biomaterials 28 (2007) 869–876, https://doi.org/10.1016/j.biomaterials.2006.09.047.
[27] K. Kataoka, T. Matsumoto, M. Yokoyama, T. Okano, Y. Sakurai, S. Fukushima, K. Okamoto, G.S. Kwon, J. Contr. Release 64 (2000) 143–153, https://doi.org/10.1016/S0168-3659(99)00133-9.
[28] X.T. Shuai, H. Ai, N. Nasongkla, S. Kim, J.M. Gao, J. Contr. Release 98 (2004) 415–426, https://doi.org/10.1016/j.jconrel.2004.06.003.
[29] Z.H. Guo, E.H. Liang, J.H. Sui, M.C. Ma, L.Q. Yang, J.W. Wang, J.S. Hu, Y. Sun, Y.J. Fan, Acta Biomater. 118 (2020) 182–195, https://doi.org/10.1016/j.actbio.2020.09.051.
[30] Y. Chen, T. Ma, P. Liu, J.L. Ren, Y.C. Li, H. Jiang, L.B. Zhang, J.T. Zhu, Small 16 (2020) 2005667, https://doi.org/10.1002/smll.202005667.